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USEPA/ORD/CPHEA Fact Sheet

In utero exposure to a mixture of the perfluoroalkyl pesticide pyrifluquinazon and dibutyl phthalate disrupt male rat reproductive development in a dose additive manner.

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Background/Overview:

Humans carry residues of multiple synthetic chemicals at any given point in time. Research has demonstrated that compounds with varying molecular mechanisms that disrupt common Key Events can act in concert to produce cumulative adverse effects. As such, one of the most pressing issues in toxicology and risk assessment is the evaluation and cumulative assessment of chemical mixtures. For example, OPPT is currently considering approaches to estimating the risk of mixtures of PFAS and mixtures of phthalates. In addition, several phthalates including the one used herein are on the TSCS high priority list for regulation.

Our research group has published several research studies on the combined effects of *in utero* exposure to chemicals that have converging Key Events within an androgen receptor signaling Adverse Outcome Pathway network. This abstract describes a new experiment that tests this hypothesis. The study design is based on a binary combination of the pyrifluquinazon (PFQ), a pesticide with perfluoroalkyl isopropyl chain, known to disrupt androgen receptor function, and dibutyl phthalate (DBP), a phthalate on the TSCA high priority list that disrupts fetal testosterone synthesis. Since these two chemicals disrupt fetal androgen signaling, albeit via different mechanisms of toxicity, we hypothesized that this binary mixture would produce permanent, adverse male reproductive effects in cumulative, dose additive manner when the mixture was administered to the pregnant rat during the period of fetal male sexual differentiation.

Relevance to EPA Program/Regional Research Needs/Priorities: This paper contributes to subproduct CSS.4.2.1.1 and is relevant to the needs of OPPT as they consider cumulative risk assessment approach for phthalates and PFAS chemicals, PFQ being a perfluoroalkyl pesticide (PFAP). Further, this information adds to the growing body of scientific information that contributes to the methods and scientific rationale for addressing issues related to chemical mixtures. In general, this body of literature indicates that dose addition should be the default model for assessing risk the risk of chemical mixtures that disrupt common signaling pathways even when they contain chemicals that disrupt development via different mechanisms of action

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Study Description: We conducted experiments with pregnant female rats to assess effects on androgen signaling and reproductive tract development in offspring at fetal, newborn, juvenile, and adult time points. Pregnant female rats were dosed during the "masculinizing window" of development, when the male reproductive tract is being programmed. We dosed the animals with vehicle control or dilutions of a mixture of two different anti-androgenic compounds including the PFAP PFQ and the phthalate DBP. The dose range of the mixture was designed so we could determine if the chemicals interacted in a dose-additive, independent, synergistic or antagonistic manner. The top dose (100% dose) contained each compound at a concentration high enough to significantly alter the reproductive system of most of the male offspring, whereas the lowest dilution contained a concentration each chemical below one known to disrupt male rat sexual differentiation. Pregnant rats were dosed from gestational day 14 to 18 with 0, 12.5, 25, 50, 75 or 100% of the top dose (Importantly, the two chemicals target two different molecular initiating events (i.e., mechanisms of action), but both ultimately disrupt androgen receptor signaling in the developing fetus.

Major Observations and Results: We found that this binary mixture of PFQ and DBP altered male reproductive tract differentiation of male reproductive tissues in a dose additive manner. Adverse outcomes including shortened anogenital distance and permanent weight deficits in multiple accessory sex tissues (LABC, seminal vesicles, glans penis) occurred at ED_{50} doses 2-fold below individual ED_{50}s.

Impact/Potential Implication of the Findings: This study demonstrates the importance of assessing the toxicity for multiple chemicals with overlapping Key Events in an AOP network. Like the results of the current study, an emerging body of information from multiple laboratories supports the hypothesis that when individual chemicals that disrupt androgen receptor signaling via multiple MIEs are administered as a mixture to pregnant female rats during the critical window of sexual differentiation, permanent adverse effects occur in the offspring at doses lower than those at which the individual chemicals produce effects in individual chemical exposures.

Findings Advancing Existing Scientific Knowledge: The present study suggests that male fetuses may be at increased risk due to cumulative exposure of the pregnant woman to multiple anti-androgenic chemicals. Moving forward, cumulative risk assessment approaches should consider grouping chemicals based on a common biological signaling pathway and these pathways can be elucidated using the AOP framework. Further, the dose addition continues to be the most accurate model for predicting mixture-based toxicological effects.

Publication Information (journal, book chapter/book) and Estimated Timelines: This abstract will be submitted to Fluoros Global 2021 since PFQ is a PFAS. The abstract will be available online during the meeting website from October 3 to 8, 2021.

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